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Investigation of the MGP promoter and exon 4 polymorphisms in patients with ischemic stroke in the Ukrainian population

Alexander V. ATAMAN[*1,] Victoria Y. GARBUSOVA[2], Yuri A. ATAMAN[3], Olga I. MATLAJ[4], Olga A. OBUCHOVA[1]

[1] *Sumy State University, Department of Physiology, Pathophysiology and Medical Biology, Sumy, Ukraine*
[2] *Sumy State University, Scientific Laboratory of Molecular Genetic Research, Sumy, Ukraine*
[3] *Sumy State University, Department of Internal Medicine, Sumy, Ukraine*
[4] *Sumy Clinical Hospital No.5, Sumy, Ukraine*
*(*author for correspondence; ataman_av@mail.ru)*

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Abstract
Matrix γ-carboxyglutamic acid protein (MGP) is a vitamin K-dependent protein playing a pivotal role in preventing arterial calcification. In the present study, we aimed to investigate the relation between three single nucleotide polymorphisms of MGP gene and ischemic stroke (IS) in the Ukrainian population. 170 IS patients and 124 healthy controls were recruited to the study. MGP SNPs were examined by PCR-RFLP methodology. The distribution of homozygous carriers of the major allelic variant, and heterozygous and homozygous minor allele variants of the T-138C MGP promoter polymorphism (rs1800802) in patients with IS was 61.2%, 31.2% and 7.6%, respectively. The corresponding distributions of the variants in the control group were 59.7%, 35.6%, 4.8%. With regard to the G-7A promoter polymorphism (rs1800801), the respective distributions were 35.9%, 48.8% and 15.3%, compared to 43.5%, 50% and 6.5% in the control group. Finally, the respective distributions according to the Thr83Ala exon 4 polymorphism (rs4236) were 39.4%, 48.8% and 11.8%, compared to 34.7%, 53.2% and 12.1% in the control group. Using logistic regression analysis, it was estimated that A/A genotype (G-7A polymorphism) was significantly (P=0.016) associated with IS (OR=2.943; 95% CI: 1.218–7.109) in the Ukrainian population. A-allele homozygotes of female sex had a risk of IS more than 7 times higher compared with carriers of G/G genotype.
***Keywords**:* Matrix Gla protein, single nucleotide polymorphism, ischemic stroke, arterial calcification, Ukrainian population.

Ukrayna popülasyonunda iskemik inme hastalarında MGP promotör ve ekzon 4 polimorfizminin araştırılması

Özet
Matris γ–karboksiglutamik asit proteini (MGP) vitamin-K bağımlı protein olup arteriyal kalsitleşmeyi önlemede önemli rol oynar. Bu çalışmada, MGP geninin üç tek nükleotit polimorfizmi (TNP) ile Ukrayna popülasyonunda iskemik inme (İİ) arasındaki ilişkiyi araştırmayı hedefledik. Çalışmaya 170 İİ hastası ve 124 sağlıklı kontrol katıldı. MGP TNP'leri PCR-RFLP metodolojisi ile test edildi. İH hastalarında T-138C MGP promoter polimorfizminin (rs1800802) majör alel varyantının homozigot taşıyıcılarının ve heterozigot ve homozigot minör alel varyantlarının dağılımları sırası ile, %61.2, %31.2 ve %7.6'dır. Kontrol grubunda ilgili varyant dağılımları %59.7, %35.6 ve %4.8'dir. G-7A promoter polimorfizminde (rs1800801) ise ilgili dağılımlar %43.5, %50 ve %6.5 olan kontrol grubu ile karşılaştırıldığında %35.9, %48.8 ve %15.3'dir. Son olarak, Thr83Ala ekzon 4 polimorfizmine (rs4236) göre dağılımlar %34.7, %53.2 ve %12.1 olan kontrol grubu ile karşılaştırıldığında %39.4, %48.8 ve %11.8'dir. Lojistik regresyon analizi kullanarak, Ukrayna popülasyonunda İİ ile A/A genotipinin (G-7A polimorfizmi) anlamlı (P=0.016) olarak ilişkili olduğu (OR=2.943; 95% CI: 1.218–7.109) tahmin edilmiştir.

Anahtar kelimeler: Matris Gla protein, tek nükleotid polimorfizmi, iskemik inme, arteriyal kalsifikasyon, Ukrayna popülasyonu.

Introduction

Ischemic stroke (IS) is, in many instances, the consequence of a thrombus forming on a ruptured atherosclerotic plaque. Extracellular matrix calcification is considered to be a novel marker of atherosclerosis and related to both coronary artery and cerebrovascular disease. It has been shown that arterial calcification in major vessel beds is associated with vascular brain disease (Bos *et al*., 2011).

Recent studies suggest that in addition to modifiable risk factors, such as hypertension, hyperlipidemia, and cigarette smoking, there is a strong genetic component to the development of arterial calcification. For instance, the heritability of the presence of coronary artery calcification has been estimated to be up to 50% (Post *et al*., 2007).

Key genes known to be involved in the regulation of the complex process of ectopic soft tissue mineralization are those acting as calcification inhibitors such as matrix γ-carboxyglutamic acid protein (MGP), osteocalcin (BGP), osteoprotegerin (Opg), and fetuin (Abedin *et al*., 2004; Doherty *et al*., 2004; Giachelli, 2004; Guzman, 2007; Weissen-Plenz *et al*., 2008). Among those, MGP, a vitamin K-dependent protein, is widely accepted as playing a pivotal role in preventing local mineralization of the vascular wall (Luo *et al*., 1997; Schurgers *et al*., 2005; Proudfoot and Shanahan, 2006). It has been shown that the anticalcifying activity of MGP depends upon the γ-carboxylation of specific glutamic acid (Glu) residues in MGP. This vitamin K-dependent reaction yields γ-carboxyglutamic acid (Gla) residues, which are then able to bind calcium (Murshed *et al*., 2004).

The human MGP gene is located on chromosome 12p (Cancela *et al*., 1990). Among the large number of identified MGP single nucleotide polymorphisms (SNPs) eight are under the most intensive investigation: two SNPs are located in exons, and six in the upstream region of the MGP gene. *In vitro* studies suggest that SNPs in MGP are associated with altered promoter activity (Herrmann *et al*., 2000; Farzaneh-Far *et al*., 2001; Kobayashi *et al*., 2004). In addition, there is some evidence that MGP SNPs are associated with arterial calcification (Herrmann *et al*., 2000; Brancaccio *et al*., 2005; Crosier *et al*., 2009), although these results are not consistent (Kobayashi *et al*., 2004; Taylor *et al*., 2005).

There are a large number of studies in which the association of varies gene polymorphisms with IS has been investigated (Kubo, 2008; Debette and Seshadri, 2009; Matarin *et al*., 2009; Wang *et al*., 2009; Low *et al*., 2011), but only in one of them the MGP SNPs were a subject of interest (del Rio-Espinola *et al*., 2010).

The purpose of the present study was to investigate the association of three MGP SNPs (T-138C, G-7A, Thr83Ala) with IS in the Ukrainian population.

Materials and methods

Study groups

The study recruited 170 IS patients (57,6% men and 42,4% women) 40 to 85 years of age (mean age [± SE] 64,7±0,7) admitted to Sumy Clinical Hospital No.5. A final diagnosis of IS was established on the basis of clinical, computed tomography and magnetic resonance imaging examinations. Each case of IS was assessed according to TOAST criteria (Adams *et al*., 1993). The patients with IS of cardioembolic origin and undetermined etiology were excluded from the study group. The control group consisted of 124 clinically healthy individuals with the absence of cardio- and cerebrovascular pathologies, as confirmed by medical history, ECG, and measurement of arterial pressure and biochemical data. The study had been previously approved by the Ethic Committee of the Medical Institute of Sumy State University. Appropriate informed consent was obtained from all patients and control subjects. The participants were unrelated Ukrainian people from the northeastern region of Ukraine. Blood sampling for genotyping was performed under sterile conditions into 2.7 ml tubes (S-Monovette [Sarstedt, Germany]) containing EDTA potassium salt as an anticoagulant, samples were frozen and stored at -20ºC.

Genotyping of SNPs

DNA for genotyping was extracted from the venous blood using commercially available kits (Isogene Lab Ltd, Russia) according to the manufacturer's protocol. To identify MGP SNPs the polymerase chain reaction (PCR) with subsequent restriction fragment length polymorphism (RFLP) analysis was performed as previously described (Garbuzova *et al.*, 2012). Briefly, specific regions of the MGP gene were amplified using pairs of specific primers.

For T-138C polymorphism (rs1800802) they were (F) 5`-AAGCATACGATGGCCAAAACTTCTGCA-3` and (R) 5`-GAACTAGCATTGGAACTTTTCCCAACC-3`; for G-7A polymorphism (rs1800801): (F) 5`-CTAGTTCAGTGCCAACCCTTCCCCACC-3` and (R) 5`-TAGCAGCAGTAGGGAGAGAGGCTCCCA-3`; for Thr83Ala polymorphism (rs4236): (F) 5`-TCAATAGGGAAGCCTGTGATG-3` and (R) 5`-AGGGGGATACAAAATCAGGTG -3`. PCR products were digested using restriction enzymes: *Bse*NI (for T-138C), *Nco*I (for G-7A), and *Eco*477 (for Thr83Ala). The restriction fragments were separated by electrophoresis and analysed on an ethidium bromide-stained 2.5% agarose gel visualized using ultraviolet transillumination.

Statistical analysis

Using the Pearson χ^2 test, allelic frequencies in healthy controls and IS patients were found to be in Hardy-Weinberg equilibrium. Statistical analysis was performed to assess the independent main and joint effects of all analyzed SNPs. To detect the strongest main effect of three MGP SNPs the logistic regression method was applied by using SPSS 17.0. A comparison of variables between the IS subgroups was performed using ANOVA. Differences were considered statistically significant with a P-value < 0.05.

Results

Genotypes of three studied MGP polymorphisms are summarized in Table 1. As shown, major allele homozygous and heterozygous, and minor allele homozygous T-138C polymorphisms of the MGP promoter were detected in 61.2%, 31.2% and 7.6% of the IS group, respectively (control group: 59.7, 35.5% and 4.8%). Analysis of the G-7A promoter polymorphism yielded respective figures of 35.9%, 48.8% and 15.3% (control group: 43.5%, 50% and 6.5%). The distribution of genotypes when analyzing Thr83Ala polymorphism (exon 4) was 39.4%, 48.8% and 11.8% in IS group (control group: 34.7%, 53.2% and 12.1).

Table 1. Genotypes of MGP polymorphisms in patients with ischemic stroke (IS) and control subjects. Data presented as n (%). A – major allele; a – minor allele

Genotype	Promoter T-138C		Promoter G-7A		Exon 4 Thr83Ala	
	Control group (n=124)	IS group (n=170)	Control group (n=124)	IS group (n=170)	Control group (n=124)	IS group (n=170)
AA	74 (59.7)	104 (61.2)	54 (43.5)	61 (35.9)	43 (34.7)	67 (39.4)
Aa	44 (35.5)	53 (31.2)	62 (50.0)	83 (48.8)	66 (53.2)	83 (48.8)
aa	6 (4.8)	13 (7.6)	8 (6.5)	26 (15.3)	15 (12.1)	20 (11.8)

The differences in the distribution of allelic variants between the control and IS groups were close to the level of statistical significance only for the G-7A promoter polymorphism (P=0,051). In women, but not in men, the differences between G-7A genotypes frequency in IS and controls were significant as shown in Table 2.

Using logistic regression analysis (Table 3), it was estimated that A/A genotype (G-7A polymorphism) was significantly (P=0.016) associated with IS (OR=2.943; 95% CI, 1.218 – 7.109). Respective analysis for male and female subjects is presented in Table 4. Women who were minor A-allele homozygotes had a risk of IS more then 7 times higher compared with female carriers of G/G genotype.

Some clinical characteristics of IS patients with various MGP genotypes are presented in Table 5. There were no differences in the studied parameters between major allele homozygotes, heterozygotes, and minor allele homozygotes for all three polymorphisms (with the exception of sex distribution for G-7A polymorphism).

Table 2. Genotypes of G-7A MGP promoter polymorphism in female and male patients with ischemic stroke (IS) and control subjects. Data presented as n (%).

Genotype	Women		Men	
	Control	IS	Control	IS
G/G	18 (40.0)	21 (29.2)	36 (45.6)	40 (40.8)
G/A	25 (55.6)	34 (47.2)	37 (46.8)	49 (50.0)
A/A	2 (4.4)	17 (23.6)	6 (7.6)	9 (9.2)
Total	45	72	79	98
P-value	0.022		0.798	

Table 3. Results of logistic regression analysis of association between MGP polymorphisms and ischemic stroke.Homozygotes by major allele were considered as a reference group. SE – standard error, OR – odds ratio, CI – confidential interval

SNP	Genotype	Coefficient of regression	SE	Wald statistic	P-value	OR	%95 CI Lower	%95 CI Upper
Promoter T-138C	T/C	-0.186	0.258	0.521	0.470	0.830	0.500	1.377
	C/C	0.382	0.526	0.527	0.468	1.465	0.522	4.107
Promoter G-7A	G/A	0.193	0.253	0.584	0.445	1.213	0.739	1.991
	A/A	1.079	0.450	5.752	0.016	2.943	1.218	7.109
Exon 4 Thr83Ala	Thr/Ala	-0.235	0.259	0.824	0.364	0.790	0.476	1.313
	Ala/Ala	-0.265	0.402	0.435	0.510	0.767	0.349	1.687

Discussion

Arterial calcification is an abnormal process that can greatly increase morbidity and mortality (Lehto *et al.*, 1996). MGP is considered one of the most relevant physiological inhibitors of soft tissue mineralization known today. In mice, targeted deletion of the MGP gene causes extensive calcification of the elastic lamellae of the abdominal aorta (Luo *et al.*, 1997). Extensive vascular calcification is also induced when γ-carboxylation of MGP is inhibited using the vitamin K-antagonist, warfarin (Price *et al.*, 1998).

In the present study, we explored association between genetic variation in the MGP gene and the risk of IS development. Analysing MGP SNPs, we found the G-7A promoter polymorphism to be associated with IS in Ukrainian population. We did not revealed statistically significant relation between the other two studied polymorphisms (T-138C, Thr83Ala) and IS.

Published data on the MGP SNPs association with MGP serum concentration and artery calcification, and the consequences of atherosclerosis (myocardial infarction in particularly) are contradictory.

Farzaneh *et al.* (2001) did not find any relationship between the G-7A polymorphism and serum MGP level in healthy persons (Netherlands), but did detect the significant association of T-138C polymorphism with above-mentioned parameter. The highest level of serum MGP was revealed in the C/C homozygotes and the lowest one – in T/T homozygotes.

In contrast to the above study, Crosier *et al.* (2009) found no association of the T-138C polymorphism with serum MGP concentration, but they showed a significant relationship between the other two polymorphisms (G-7A, Tht83Ala) and serum MGP levels in the healthy men and women (USA). In minor allele homozygotes, the serum MGP concentration was the lowest, in major allele homozygotes the highest, in heterozygotes the intermediate values were registered.

In the same study, it was shown that all three MGP SNPs (T-138C, G-7A, Thr83Ala) are related to the coronary artery calcification (CAC) in men, but not in women (Crosier *et al.*, 2009).

Table 4. Logistic regression analysis of association between G-7A MGP promoter polymorphism and ischemic stroke in male and female subjects. OR – odds ratio, CI – confidential interval

Sex	Allele	OR (CI)	P-value
Women	A/A vs. G/G	7.286 (1.479-35.895)	0.015
	G/A vs. G/G	1.166 (0.516-2.632)	0.712
Men	A/A vs. G/G	1.350 (0.437-4.166)	0.602
	G/A vs. G/G	1.192 (0.641-2.217)	0.579

Table 5. Clinical characteristics of ischemic stroke patients with respect to genotypes. Data are mean ± SE.

	A/A	A/a	a/a	P
T-138C polymorphism				
n	104	53	13	
Age, years	65.4±0.92	63.0±1.39	64.7±2.13	0.288
Gender, M/F	58/46	35/18	5/8	0.162*
BMI (M), kg/m^2	27.8±0.56	27.4±0.64	28.1±1.22	0.862
BMI (F), kg/m^2	29.1±0.74	29.4±0.93	29.0±1.17	0.789
Systolic BP, mmHg	168±2.9	165±3.6	168±9.8	0.780
Diastolic BP, mmHg	96±1.7	94±1.8	93±3.8	0.628
Fasting glucose, mmol/L	5.9±0.15	5.9±0.2	6.1±0.53	0.916
G-7A polymorphism				
n	61	83	26	
Age, years	63.0±1.15	65.3±1.04	66.8±2.09	0.164
Gender, M/F	40/21	49/34	9/17	0.026*
BMI (M), kg/m^2	27.2±0.45	27.9±0.68	28.6±1.53	0.536
BMI (F), kg/m^2	28.3±0.8	29.9±0.86	28.2±1.13	0.315
Systolic BP, mmHg	167±3.7	167±3.3	167±5.2	0.996
Diastolic BP, mmHg	97±2.0	94±1.8	97±2.4	0.593
Fasting glucose, mmol/L	5.8±0.18	6.0±0.17	6.2±0.35	0.481
Thr83Ala polymorphism				
n	67	83	20	
Age, years	65.1±1.2	64.4±1.0	64.7±2.1	0.912
Gender, M/F	44/23	45/38	9/11	0.176*
BMI (M), kg/m^2	27.6±0.47	27.6±0.67	28.3±1.9	0.891
BMI (F), kg/m^2	29.4±0.84	28.6±0.78	29.9±1.53	0.668
Systolic BP, mmHg	163±3.7	171±3.0	163±6.6	0.252
Diastolic BP, mmHg	95±1.8	96±1.7	95±4.4	0.812
Fasting glucose, mmol/L	5.9±0.2	6.0±0.17	5.9±0.3	0.859

In some studies, MGP polymorphisms were also shown to be associated with arterial calcification and myocardial infarction (MI) (Herrmann *et al*., 2000; Brancaccio *et al*., 2005), while in others (Kobayashi *et al*., 2004; Taylor *et al*., 2005) no association between MGP SNPs and cardiovascular events was found. Moreover, in the studies in which such associations were reported, the relationship between the type of MGP polymorphism and arterial calcification was different. For example, in the AXA study, the minor alleles -7A and 83Ala were associated with increased femoral artery calcification (Herrmann *et al*., 2000), while in the above-mentioned study by Crosier et al. (2009), the same alleles were linked to a decreased level of CAC.

It should be noted that the majority of studies cited here was devoted to the relation of MGP to CAC and MI. As to cerebral artery atherosclerosis and its severe events such as IS, the role of arterial calcification in this disease and the association of MGP with cerebrovascular pathology were the subject of investigation and discussion only in a few publications. In particular, Bos *et al*. (2011) established a close relationship between calcification in the various vessel beds outside the brain and imaging markers of vascular brain disease. Calcification in each vessel bed was shown to be associated with the presence of cerebral infarcts and with larger volume of white matter lesions (WMLs). The most prominent associations were found between the intracranial carotid calcification and WML volume and between the extracranial carotid calcification and infarcts.

Acar *et al.* (2012) studied a relationship of serum MGP levels to the development of intracerebral hemorrhages (ICH) and found that in patients with ICH, serum MGP concentration was much lower than in control group. Moreover, in the non-survivors, the serum MGP levels were statistically significantly lower in comparison to the survivors. According to the authors, measurement of this parameter may be of value to estimate mortality.

At present, there are only a few publications concerning relation of the MGP SNPs to cerebrovascular disease. Analysing 236 polymorphisms, del Rio-Espinola *et al.* (2010) showed that only two of them (G-7A of MGP and T-1C of CD40) were related to the brain vessel reocclusion after fibrinolysis in IS patients. In our study, it was shown that the G-7A polymorphism of MGP was associated with IS. In our previous investigation (Harbusova *et al*., 2011), this variant of the MGP promoter polymorphism was found to be in association with the acute coronary syndrome (ACS). Minor allele homozygotes (A/A) had significantly higher risk of ACS as well as IS. This could means that there are some common mechanisms of pathogenesis in both ACS and IS concerning to MGP. Those may be atherosclerosis, arterial calcification, and thrombosis.

The relation of MGP to blood vessels calcification is well known (see above). With respect to coagulation and thrombi formation, it can be suggested that MGP is somehow connected with these processes (Krueger *et al*., 2009). Such an assumption is based on the fact that MGP belongs to vitamin K-dependent proteins, a large number of which are procoagulants (prothrombin, factor V, etc) and can influence blood clotting and thrombi formation in the coronary and cerebral arteries. In some papers (Wallin *et al*., 2008), an antagonistic relationship between calcification and coagulation is discussed. Therefore, MGP can be considered as a connecting link between these two processes. Certainly, this assumption requires experimental as well as clinical proofs, and research in this direction should be continued.

References

Abedin M, Tintut Y, Demer LL. Vascular calcification. Mechanisms and clinical ramifications. *Arterioscler Thromb Vasc Biol*. 24: 1161-1170, 2004.

Acar A, Cevik MU, Arıkanoglu A, Evliyaoglu O, Basarılı MK, Uzar E, Ekici F, Yucel Y, Tasdemir N. Serum levels of calcification inhibitors in patients with intracerebral hemorrhage. *Int J Neurosci*. 122: 227-232, 2012.

Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke*. 24: 35-41, 1993.

Bos D, Ikram MA, Elias-Smale SE, Krestin GP, Hofman A, Witteman JC, van der Lugt A, Vernooij MW. Calcification in major vessel beds relates to vascular brain disease. *Arterioscler Thromb Vasc Biol*. 31: 2331-2337, 2011.

Brancaccio D, Biondi ML, Gallieni M, Turri O, Galassi A, Cecchini F, Russo D, Andreucci V, Cozzolino M. Matrix Gla protein gene polymorphisms: clinical correlates and cardiovascular mortality in chronic kidney disease patients. *Am J Nephrol*. 25: 548-552, 2005.

Cancela L, Hsiehg CL, Francket U, Price PA. Molecular structure, chromosome assignment, and promoter organization of the human matrix Gla protein gene. *J Biol Chem*. 265: 15040-15048, 1990.

Crosier MD, Booth SL, Peter I, Dawson-Hughes B, Price PA, O'Donnell CJ, Hoffmann U, Wiilliamson MK, Ordovas JM. Matrix Gla protein polymorphisms are associated with coronary artery calcification. *J Nutr Sci Vitaminol*. 55: 59-65, 2009.

Debette S, Sechardi S. Genetics of atherothrombotic and lacunare stroke. *Circ Cardiovasc Gen*. 2: 191-198, 2009.

del Río-Espínola A, Fernández-Cadenas I, Rubiera M, Quintana M, Domingues-Montanari S, Mendióroz M, Fernández-Morales J, Giralt D, Molina CA, Alvarez-Sabín J, Montaner J. CD40-1C>T polymorphism (rs1883832) is associated with brain vessel reocclusion after fibrinolysis in ischemic stroke. *Pharmacogenomics*. 11: 763-772, 2010.

Doherty TM, Fitzpatrick LA, Inoue D, Qiao JH, Fishbein MC, Detrano RC, Shah PK, Rajavashisth TB. Molecular, endocrine, and genetic mechanisms of arterial calcification. *Endocrine Rev*. 25: 629-672, 2004.

Farzaneh-Far A, Davies JD, Braam LA, Spronk HM, Proudfoot D, Chan SW, O'Shaughnessy KM, Weissberg PL, Vermeer C, Shanaham CM. A polymorphism of the human matrix γ-carboxyglutamic acid protein promoter alters binding of an activating protein-1 complex and is associated with altered transcription and serum levels. *J Biol Chem*. 276: 32466-32473, 2001.

Garbuzova VYu, Gurianova VL, Story DA, Dosenko VE, Parkhomenko AN, Ataman AV Association of matrix Gla protein gene allelic polymorphisms ($G^{-7}\rightarrow A$, $T^{-138}\rightarrow C$ and $Thr_{83}\rightarrow Ala$) with acute coronary syndrome in the Ukrainian population. *Exp Clin Cardiol*. 17: 30-33, 2012.

Giachelli CM. Vascular calcification mechanisms. *J Am Soc Nephrol*. 15: 2959-2964, 2004.

Guzman RJ. Clinical, cellular, and molecular aspects of arterial calcification. *J Vasc Surg*. 45 (Suppl A): A57-A63, 2007.

Harbusova VYu, Hurianova VL, Parkhomenko OM, Dosenko VE, Ataman OV. The frequency of allelic polymorphism of matrix Gla-protein gene in acute coronary syndrome patients. *Fiziol Zh*. 57: 16-24, 2011.

Herrmann SM, Whatling C, Brand E, Nikaud V, Gariepy J, Simon A, Evans A, Ruidavets LB, Arveiler D, Luc G, Tiret L, Henney A, Cambien F. Polymorphisms of the human matrix Gla protein (MGP) gene, vascular calcification, and myocardial infarction. *Arterioscler Thromb Vasc Biol*. 20: 2386-2393, 2000.

Kobayashi N, Kitazawa R, Maeda S, Schurgers LJ, Kitazawa S. T-138C polymorphism of matrix Gla protein promoter alters its expression but is not directly associated with atherosclerotic vascular calcification. *Kobe J Med Sci*. 50: 69-81, 2004.

Krueger T, Westenfeld R, Schurgers LJ, Brandenburg VM. Coagulation meets calcification: The vitamin K system. *Int J Artif Organs*. 32: 67-74, 2009.

Kubo M. Genetic risk factors of ischemic stroke identified by a genome-wide association study. *Brain Nerve*. 60: 1339-1346, 2008.

Lehto S, Niskanen L, Suhonen M, Rönnemaa T, Laasko M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 16: 978-988, 1996.

Low HQ, Chen CP, Kasiman K, Thalamuthu A, Ng SS, Foo JN, Chang HM, Wong MC, Tai ES, Liu J. A comprehensive association analysis of homocysteine metabolic pathway genes in Singaporean Chinese with ischemic stroke. *PLoS One*. 6: e24757, 2011.

Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix Gla protein. *Nature*. 386: 78-81, 1997.

Matarin M, Brown WM, Dena H, Britton A, De Vrieze FW, Brott TG, Brown RD, Worrall BB, Case LD, Chanock SJ, Metter EJ, Ferruci L, Gamble D, Hardy JA, Rich SS, Singleton A, Meschia JF. Candidate gene polymorphisms for ischemic stroke. *Stroke*. 40: 3436-3442, 2009.

Murshed M, Schinke T, McKee MD, Karsenty G. Extra cellular matrix mineralization is regulated locally: different roles of two Gla-containing proteins. *J Cell Biol*. 165: 625-630, 2004.

Post W, Bielak LF, Ryan KA, Cheng YC, Shen H, Rumberger JA, Sheedy PF, Shuldiner AR, Peyser PA, Mitchell BD. Determinants of coronary artery and aortic calcification in the Old Order Amish. *Circulation*. 115: 717-724, 2007.

Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. *Arterioscler Thromb Vasc Biol*. 18: 1400-1407, 1998.

Proudfoot D, Shanahan CM. Molecular mechanisms mediating vascular calcification: role of matrix Gla protein. *Nephrology (Carlton)*. 11: 455-461, 2006.

Schurgers LJ, Teunissen KJF, Knapen MHJ, Kwaijtaal M, van Diest R, Appels A, Reutelingsperger CP, Cleutjens JPM, Vermeer C. Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein. *Arterioscler Thromb Vasc Biol*. 25: 1629-1633, 2005.

Taylor BC, Schreiner PJ, Doherty TM, Fornage M, Carr JJ, Sidney S. Matrix Gla protein and osteopontin genetic associations with coronary artery calcification and bone density: the CARDIA study. *Hum Genet*. 116: 525-528, 2005.

Wallin R, Schurgers L, Wajih N. Effects of the blood coagulation vitamin K as an inhibitor of arterial calcification. *Thromb Res*. 122: 411-417, 2008.

Wang X, Cheng S, Brophy VH, Erlich HA, Mannhalter C, Berger K, Lalouschek W, Browner WS, Shi Y, Ringelstein EB, Kessler C, Luedemann J, Lindpaintner K, Liu L, Ridker PM, Zee RY, Cook NR. A meta-analysis of candidate gene polymorphisms and ischemic stroke in 6 study populations: association of lymphotoxin-alpha in nonhypertensive patients. *Stroke*. 40: 683-695, 2009.

Weissen-Plenz G, Nitschke Y, Rutsch F. Mechanisms of arterial calcification: spotlight on the inhibitors. *Adv Clin Chem*. 46: 263-293, 2008.